

Protocol for ligation

Test the concentration of the DNA sample(s);

Pipet the following into a 0.2ml microfuge tube:

Linearized vector DNA: around 100ng

Insert DNA (at 3:1 molar excess over vector): variable

10x ligation buffer: 1uL

T4 DNA Ligase(NEB): 1uL

ddwater: up to 10 ul

Vortex thoroughly and spin briefly to collect drops;

Incubate the mixture at 16 degree for 60-120 min;

Use the ligation mixture for transformation;